Both Cell Fusion and Transdifferentiation Account for the Transformation of Human Peripheral Blood CD34-Positive Cells Into Cardiomyocytes In Vivo

Sui Zhang, MD, PhD; Dachun Wang, MD; Zeev Estrov, MD; Sean Raj; James T. Willerson, MD; Edward T.H. Yeh, MD

Background—Adult human peripheral blood CD34-positive (CD34⁺) cells appear to transform into cardiomyocytes in the injured hearts of severe combined immunodeficient mice. It remains unclear, however, whether the apparent transformation is the result of transdifferentiation of the donor stem cells or of fusion of the donor cell with the cardiomyocyte of the recipients.

Methods and Results—We performed flow cytometry analyses of cells isolated from the hearts of mice that received human CD34⁺ cells. Human HLA-ABC antigen and cardiac troponin T or Nkx2.5 were used as markers for cardiomyocytes derived from human CD34⁺ cells, and HLA-ABC and VE-cadherin were used to identify the transformed endothelial cells. The double-positive cells were collected and interphase fluorescence in situ hybridization was used to detect the expression of human and mouse X chromosomes in these cells. We found that 73.3% of nuclei derived from HLA⁺ and troponin T⁺ or Nkx2.5⁺ cardiomyocytes contain both human and mouse X chromosomes and 23.7% contain only human X chromosome. In contrast, the nuclei of HLA⁺, troponin T⁺ cells contain only mouse X chromosomes. Furthermore, 97.3% of endothelial cells derived from CD34⁺ cells contained human X chromosome only.

Conclusions—Thus, both cell fusion and transdifferentiation may account for the transformation of peripheral blood CD34⁺ cells into cardiomyocytes in vivo. (Circulation. 2004;110:3803-3807.)

Key Words: antigens, CD34 ■ stem cell ■ cardiomyocyte ■ cell fusion

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ome cells of different origins have been observed to develop into a variety of cell types including cardiomyocytes, hepatocytes, and epithelial cells of the gastrointestinal tract after transplantation. The nature of this transformation, however, is unclear. Different research groups, using different detection methods in different experimental settings, have proposed different mechanisms for the apparent transformation of stem cells into cells of a variety of tissues. Some investigators attribute this transformation to the transdifferentiation potential of stem cells. Others have demonstrated that this apparent transformation is a result of cell fusion. It is widely known that stem cells of various origins can develop into cardiomyocytes. Many investigators have demonstrated that bone marrow hematopoietic stem cells, embryonic stem cells, adult mesenchymal stem cells, hematopoietic stem cells in peripheral blood, cardiac progenitor cells, and adult cardiac stem cells home to the heart and transform into cardiomyocytes in vitro and in vivo. Most investigators attribute the phenotypic conversion to transdifferentiation. Recently, one report demonstrated that cell fusion is responsible for the transformation. We previously reported that human peripheral blood CD34-positive (CD34⁺) cells may develop into cardiomyocytes, vascular smooth muscle cells, and endothelial cells in the injured hearts of severe combined immunodeficient (SCID) mice. Because the mechanism of the phenotypic transformation is important in evaluating the clinical feasibility of potential cellular therapy for heart failure, we have addressed the question of whether transformation of the CD34⁺ cells into cardiomyocytes is the result of transdifferentiation of the injected stem cells or fusion between donor cells and the cardiomyocytes of the recipient.

Methods

Animals

Female SCID mice (C3H, Jackson Laboratory, Bar Harbor, Maine) weighing 14 to 18 g were used in the study. The Institutional Animal Care and Use Committees of the University of Texas Health Science Center approved the use of animals in the study. The Institutional Review Board at the University of Texas-Houston Health Science Center approved the use of cells isolated from human donors. The study was conducted in accordance with the standards of the Declaration of Helsinki.

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Peripheral Blood
pore size of 70
Interphase Fluorescence In Situ Hybridization
The collected double-positive cells were spun onto a slide and fixed immediately with 3:1 methanol:acetic acid solution for 30 minutes. To quench the residual fluorescence from cell sorting, the slides were exposed to white light for 120 hours at 4°C. Complete diminishment of the residual fluorescence was confirmed by examination under an epifluorescence microscope (Nikon Eclipse TE2000U). Slides were briefly fixed in 3:1 methanol:acetic acid again and were predenatured, dehydrated, and denatured according to the manufacturer’s protocol. Slides were hybridized with a fluorescein isothiocyanate–conjugated DNA probe for mouse X chromosomes (ID Labs) and a PE-conjugated probe for human X chromosomes (Qiagen) overnight at 37°C in a humidified chamber. After posthybridization wash, slides were counterstained with 4',6-diamidino-2-phenylindole (0.02 μg/mL) and examined with an epifluorescence microscope (Nikon Eclipse TE2000U).

Results
Engraftment of Human CD34+ Cells in the Heart
To evaluate engraftment of the CD34+ cells in the heart and transformation of these cells into the cardiomyocytes, we examined isolated cells by FACS analysis using specific antibodies against HLA-ABC, a surface marker for human cells, and cardiac troponin T, a cardiomyocyte-specific marker. HLA+ cells were detected in all 4 mice examined. Approximately 2% (2.0 ± 0.4%) of the total cells from the heart were human HLA+ (Figure 1A), whereas cells from control mice with induced MI but not injected with CD34+ cells, were all HLA− (data not shown). FACS analysis of heart cells double-stained with antibodies against HLA and cardiac troponin T (Figure 1B) and with antibodies against HLA and Nkx2.5 (Figure 1C) demonstrated that ~1% of cells were double-positive, suggesting that these cardiomyocytes originated from the transplanted human cells. To ensure that immunostaining accurately reflects the genotype of isolated cells, DNA from the sorted double-positive cells and the cells stained only with anti–cardiac troponin was extracted for PCR detection of the human HLA-B gene. HLA-B fragment was amplified only in double-positive cells (Figure 1D). Thus, it is clear that the double-positive cells are of human origin.

Interphase Fluorescence In Situ Hybridization Analysis of Cardiomyocytes Developed From Transplanted CD34+ Cells
The population of double-positive cells was collected by cell sorting and examined with fluorescence in situ hybridization (FISH) analysis, in which specific probes for human and mouse X chromosomes were used simultaneously. The specificity of the probes was tested in mouse heart cells and human Hela cells by incubating these cells with both probes, and we confirmed that these 2 probes did not cross-react (Figure 2A). In the nuclei derived from cells that were troponin T− but HLA+, only mouse X chromosomes were detected (Figure 2B). Because the recipient mice were female, 2 X chromosomes were observed in each nucleus. In troponin T+ and HLA+ cells, ~70% of the nuclei contained both human and mouse X chromosomes (Table 1, Figure 2C), suggesting that cell fusion had occurred. Because the human...
donor is male, 1 human X chromosome was paired with 2 mouse X chromosomes in each nucleus (Figure 2C); however, ~30% of the nuclei of troponin T, HLA cells contained only human X chromosomes (Table 1, Figure 2D), suggesting that transdifferentiation of CD34 cells has also taken place. Analysis was also performed on HLA and Nkx2.5 sorted cells in 3 mice and similar findings were obtained (Table 1); however, only human X chromosomes were detected in ~97% of cells stained positive to both anti–HLA-ABC and anti–VE-cadherin (Table 2).

Discussion
Different research groups have reported contradictory results on the nature of the apparent transformation of stem cells into cardiomyocytes. The discrepancy could have resulted from differences in the sources of stem cells, the approaches used to distinguish fusion from transdifferentiation, and the experimental settings.

In our study, we used an animal model in which SCID mice were transplanted with human peripheral blood CD34 cells after the mouse hearts were injured by experimental MI. The specific antigen (human HLA-ABC) of the donor cells allowed us to track these cells accurately and rapidly using immunodetection methods, such as FACS analysis. Instead of evaluating tissue sections of the heart, we were able to examine quantitatively the entire heart and collect populations of cells of interest by using FACS sorting. Our approach, therefore, enables us to focus on an individual cell.
Our results indicated that both cell fusion and transdifferentiation are present in the model of ischemia reperfusion injury followed by the injection of bone marrow cells. We observed the presence of both cell fusion and transdifferentiation in animal models.17,18 The results of these studies are consistent with the findings of Alvarez-Dolado et al.10 Our study is consistent with the findings of Oh et al11 in that we did not observe transformation of bone marrow stem cells into cardiomyocytes in animal models.17,18 The reason for the inability to detect cardiomyocyte transformation is not clear, but it could be related to differences in cell origin, cell preparation, and detection methodology.

In conclusion, our results suggest that human peripheral blood CD34+ cells develop into cardiomyocytes in the injured hearts of SCID mice through both cell fusion and transdifferentiation. A number of studies have already demonstrated the application of human bone marrow mesenchymal stem cells to repair damaged myocardium.19–21 Our studies, thus, provide mechanistic insight on how human stem cells can transform into cardiomyocytes in vivo.

TABLE 2. FISH Analysis of Nuclei From Cells Double-Stained With Anti–HLA-ABC and Anti–VE-Cadherin Mice Transplanted With Human Peripheral Blood CD34+ Cells

<table>
<thead>
<tr>
<th>HLA+ and VE-Cadherin+</th>
<th>X Chromosome in Nuclei</th>
<th>Human and Mouse</th>
<th>Total Nuclei Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse 1, n (%)</td>
<td>1 (1)</td>
<td>101 (97.1)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Mouse 2, n (%)</td>
<td>0 (0)</td>
<td>127 (97.7)</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>Mouse 3, n (%)</td>
<td>1 (0.8)</td>
<td>119 (98.4)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Mouse 4, n (%)</td>
<td>0 (0)</td>
<td>136 (99.3)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Mouse 5, n (%)</td>
<td>5 (3.6)</td>
<td>131 (95)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Mouse 6, n (%)</td>
<td>3 (2)</td>
<td>141 (96)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Mean±SE</td>
<td>1.2±0.6%</td>
<td>97.3±0.6%</td>
<td>1.5±0.2%</td>
</tr>
</tbody>
</table>

References


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